John von Neumann Institute for Computing

Fast and Accurate Structure-Based Calculation of Folding Free Energies and Binding Affinities

C. M. Becker, A. Benedix, B. L. de Groot,
A. Caflisch, R. A. Böckmann

published in

From Computational Biophysics to Systems Biology (CBSB08),
Proceedings of the NIC Workshop 2008,
Ulrich H. E. Hansmann, Jan H. Meinke, Sandipan Mohanty,
Walter Nadler, Olav Zimmermann (Editors),
John von Neumann Institute for Computing, Jülich,
NIC Series, Vol. **40**, ISBN 978-3-9810843-6-8, pp. 161-164, 2008.

http://www.fz-juelich.de/nic-series/volume40

Fast and Accurate Structure-Based Calculation of Folding Free Energies and Binding Affinities

Caroline M. Becker[1], Alexander Benedix[1], Bert L. de Groot[2], Amedeo Caflisch[3], and Rainer A. Böckmann[1]

[1] Theoretical and Computational Membrane Biology, Center for Bioinformatics Saar, 66041 Saarbrücken, Germany
E-mail: {c.becker,benedix,rainer}@bioinformatik.uni-saarland.de

[2] Computational Biomolecular Dynamics Group, Max-Planck Institute for Biophysical Chemistry, Am Fassberg 11, 37077 Göttingen, Germany

[3] Department of Biochemistry, University of Zürich, Winterthurerstrasse 190, 8057 Zürich, Switzerland

We present a fast structure-based method for the prediction of folding free energies and protein-protein binding affinities, including full flexibility. The method replaces molecular dynamics simulations by a fast generation of alternative protein-protein conformations based on geometric considerations only. The energy function is based on physical chemistry and an efficient continuum solvent approach. The correlation between experimental and predicted free energies obtained for a dataset including almost 600 mutants and more than 350 protein-protein complex mutants is ≈ 0.8 with a standard deviation of 1 kcal/mol[1]. Owing to its velocity and its predictive power, the method can be applied to complete mutational scans.

1 Introduction

Protein-protein interactions are involved in most processes in the cell and are therefore an important target in pharmaceutical research. By inhibition or increase of protein-protein complex formation the activity of many processes can be influenced. However, the directed design of protein interaction surfaces with defined properties involves large-scale mutational scans. Because of their inherent computational complexity, these are precluded by the most rigorous and accurate methods in this context, the free energy perturbation (FEP) and the thermodynamic integration (TI) methods. These make use of a physical effective energy function (force field). In these methods, also termed computational alchemy, integration over the free energy gradient with respect to a perturbation parameter yields the free energy difference between two states. Both methods require molecular dynamics simulations at least in the nanosecond time range to reach sufficient convergence, using explicit solvation. FEP and TI probably work best for conservative single point mutations, but have also been successfully applied to calculate absolute binding free energies. Hybrid methods like the molecular mechanics/Poisson Boltzmann surface area (MM/PBSA[2]) method combine the calculation of molecular mechanics free energies with continuum solvent calculations.
Here, we developed a fast method, both to estimate the effect of mutations on the folding free energy of isolated proteins as well as on the protein-protein binding affinity. This fast structure-based prediction makes a systematic computational mutagenesis of protein interfaces feasible and thus will allow for a thorough analysis of the binding characteristics of

protein complexes, a pre-requisite for the design of mutants with specified properties, or for the determination of protein-ligand binding affinities. The proposed method combines a fast generation of conformations based on geometrical constraints only with a physical effective free energy function.

2 Methods

In our method[1], the conformational flexibility of proteins or protein-protein complexes is treated by CONCOORD[3]: Interactions in the (mutated) crystal input structure are analyzed and translated into geometrical constraints. Type-dependent margins are added on different interaction classes. Starting from random coordinates, the structure is iteratively rebuilt fulfilling all geometrical constraints. In this way, multiple independent structures are generated where each conformation is uncorrelated with previously generated ones[3]. For the prediction of free energies based on these (energy-minimized) structural ensembles, 300 structures were generated. The free energy is approximated by an energy function similar to the MM/PBSA approach[2]:

$$\Delta G = \Delta G_{\text{solvation}} + \Delta G_{\text{electrostatic}} + \Delta G_{\text{MM}} + \Delta G_{\text{entropy}} \; . \tag{1}$$

Figure 1 shows the used thermodynamic cycle to compute the effect of mutations on protein-protein binding affinity. The change in affinity is computed according to

$$\begin{aligned} \Delta\Delta G &= \Delta G^{\text{bind}}_{\text{Mutant}} - \Delta G^{\text{bind}}_{\text{Wildtype}} \\ &= \Delta G^{\text{mutate}}_{\text{complex}} - \Delta G^{\text{mutate}}_{\text{single}} \; . \end{aligned} \tag{2}$$

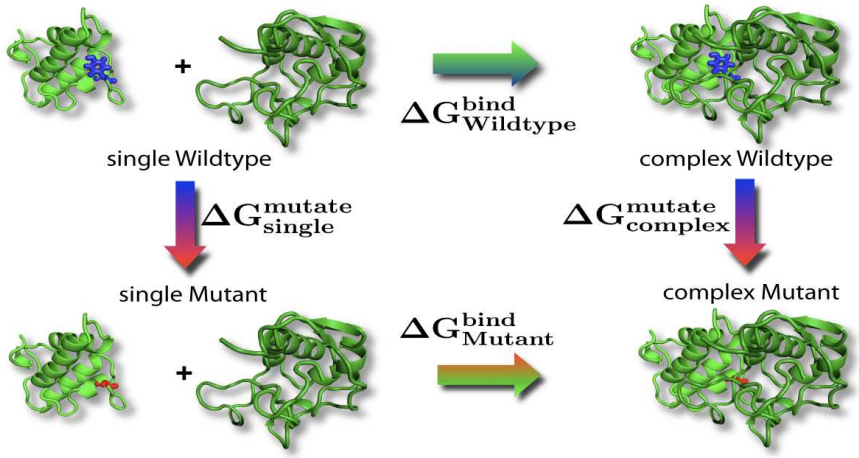

Figure 1. Thermodynamic cycle used to estimate the effect of a mutation on the protein-protein binding affinity.

3 Results

Figure 2 displays the flexibility of the response regulator protein of bacterial chemotaxis, CheY, upon the D12A mutation (highlighted in green). Especially the flexibility of neighboured residues is significantly enhanced for the mutant (lower right). The overall correlation achieved for the folding stability of almost 600 mutants is 0.75 with a standard deviation of less than 1 kcal/mol. For protein-protein binding, the obtained correlation is ≈ 0.8 with a standard deviation of 1.2 kcal/mol.

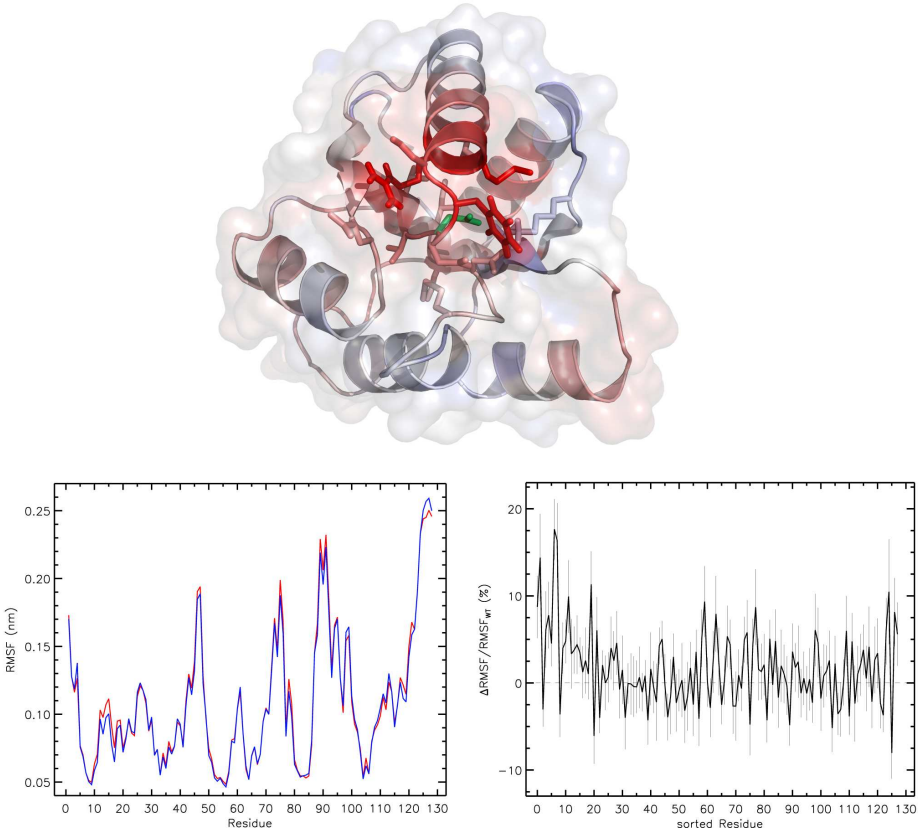

Figure 2. Color-coded change in flexibility of the response regulator protein of bacterial chemotaxis, CheY, upon the mutation D12A. The change in flexibility is additionally shown as a function of the residue number and as a function of the distance of the respective residues to the mutation site.

Acknowledgments

AB, CB, BLG, and RAB acknowledge support by the Deutsche Forschungsgemeinschaft (Grant Bo 2963/1-1, GR 2079/4-1, and BIZ 4/1). Part of the work was supported by the Swiss National Center for Competence in Research in Structural Biology (RAB and AC).

References

1. A. Benedix, C. M. Becker, B. L. de Groot, A. Caflisch, R. A. Böckmann, *CC/PBSA: Structure-based prediction of free energy and flexibility of mutants*, in preparation.
2. P. A. Kollman et al., *Calculating structures and free energies of complex molecules: combining molecular mechanics and continuum methods*, Acc. Chem. Res. **33**, 889–897, 2000.
3. B. L. de Groot, D. M. F. van Aalten, R. M. Scheck, A. Amadei, G. Vriend, H. J. C. Berendsen, *Prediction of protein conformational freedom from distance constraints.*, Proteins **29**, 240–251, 1997.